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Drinking Water Nitrate and Health –Recent Findings and Research Needs

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Abbreviations: CI, Confidence Interval; HNO_2, nitrous acid; MetHb, methemoglobin; MCL, maximum contaminant level; mg/L, milligrams per liter; nitrate-N, nitrate-nitrogen; NO, nitric oxide; NO_2, nitrogen dioxide; N_2O_3, dinitrogen trioxide; NOC, N-nitroso compounds; NTD, neural tube defect; OR, odds ratio; SB, spontaneous abortion; USEPA, United States Environmental Protection Agency; WHO, World Health Organization;

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Abstract

Human alteration of the nitrogen cycle has resulted in steadily accumulating nitrate in our water resources. The U.S. maximum contaminant level and World Health Organization guideline for nitrate in drinking water were promulgated to protect infants from developing methemoglobinemia, an acute condition. Some scientists have recently suggested that the regulatory limit for nitrate is overly conservative; however, they have not thoroughly considered chronic health outcomes. In August 2004, a symposium on drinking water nitrate and health was held at the International Society for Environmental Epidemiology meeting to evaluate nitrate exposures and associated health effects in relation to the current regulatory limit. The contribution of drinking water nitrate towards endogenous formation of N-nitroso compounds was evaluated with a focus towards identifying subpopulations with increased rates of nitrosation. Adverse health effects may be the result of a complex interaction of the amount of nitrate ingested, the concomitant ingestion of nitrosation cofactors and precursors, and specific medical conditions that increase nitrosation. Workshop participants concluded that more experimental studies are needed and that a particularly fruitful approach may be to conduct epidemiological studies among susceptible subgroups with increased endogenous nitrosation. The few epidemiologic studies that have evaluated intake of nitrosation precursors and/or nitrosation inhibitors, have observed elevated risks for colon cancer and neural tube defects associated with drinking water nitrate concentrations below the regulatory limit. The role of drinking water nitrate exposure as a risk factor for specific cancers, reproductive outcomes, and other chronic health effects needs to be more thoroughly studied before changes to the regulatory level for nitrate in drinking water can be considered.

Introduction

Humans have altered the nitrogen cycle dramatically over the last half century and as a result nitrate is steadily accumulating in our water resources. Globally, human nitrogen production increased rapidly since 1950 and it currently exceeds nitrogen fixed by natural sources by about 30% (Fields 2004). This compares to pre-1950 human inputs which were a small fraction of the input from natural sources (Lambert and Driscoll 2003). Fertilizer is the largest contributor to anthropogenic nitrogen worldwide; other major sources include animal and human waste, nitrogen oxides from utilities and automobiles, and leguminous crops that fix atmospheric nitrogen (Fields 2004). These organic and inorganic sources of nitrogen are transformed to nitrate by mineralization, hydrolysis, and bacterial nitrification. Under reducing conditions, nitrate can be biologically transformed to nitrogen gas through denitrification. Nitrate that is not taken up by plants or denitrified migrates to streams and ground water.

The U.S. EPA maximum contaminant level (MCL) for nitrate in drinking water of 10 mg/L nitrate-nitrogen (nitrate-N) (equivalent to 45 mg/L as nitrate) and the World Health Organization (WHO) guideline of 50 mg/L as nitrate (equivalent to 11 mg/L as nitrate-N) were promulgated to protect against methemoglobinemia or "blue baby syndrome", to which infants are especially susceptible. The regulatory level is usually met for public water supplies, which are routinely monitored. Much less is known about private wells which in the U.S. are usually required to be tested only when the well is constructed or when the property is sold. Recently, it has been suggested that the regulatory level for nitrate in drinking water is overly conservative (Avery 1999; L'hirondel and L'hirondel

2002). However, this discussion of the regulatory level has not thoroughly considered studies of other chronic health effects including cancer, adverse reproductive outcomes, and diabetes. Whereas a causal role for nitrate in these other health outcomes is not conclusive, recent studies that indicate possible adverse effects at nitrate levels below the MCL are of concern (Ward et al., 1996; Weyer et al., 2001; DeRoos et al., 2003; Brender et al., 2004a).

In recognition of the widespread contamination of drinking water sources by nitrate and the potential for additional health effects besides methemoglobinemia, a Symposium titled "Drinking Water Nitrate and Health: Recent Findings and Research Needs" took place at the annual meeting of the International Society for Environmental Epidemiology (August 1-4, 2004, New York, New York). Invited experts presented results from recent unpublished studies and summarized the state of knowledge on exposure and health effects of drinking water nitrate with a focus on cancer and adverse reproductive outcomes. This article summarizes the symposium discussions and recommends promising areas for future research. Specifically, we discuss the epidemiologic evidence for drinking water nitrate and risk of specific cancers, adverse reproductive outcomes, and other health outcomes, in the context of the current regulatory limit for nitrate in drinking water.

Nitrate levels in groundwater and water supplies

Nitrate is the most common chemical contaminant in the world's groundwater aquifers (Spalding and Exner 1993). An estimated 42% of the US population uses ground water as their drinking water supply (Hutson et al. 2004). In the United States, total nitrogen in

streams and nitrate in ground water are highest in agricultural areas, followed by urban areas and areas with mixed land use (figure 1). The most recent data indicate that about 22 percent of domestic wells in agricultural areas in the U.S. exceeded the MCL (U.S. Geological Survey unpublished data). In contrast, three percent of public supply wells in major aquifers (typical sources for public water supplies) exceed the MCL (U.S. Geological Survey unpublished data).

The exposure picture is similar in the European Union. Public water supplies are largely below the WHO guideline; however, in some countries private wells in rural areas have elevated nitrate concentrations reaching 10-15 times the recommended level (European Environment Agency 2003). Overall, nitrate levels exceeded the guideline in about one-third of the groundwater bodies for which data were available (European Environment Agency 2003). Several eastern European countries report high levels of nitrate contamination in a large proportion of private wells; for example, in Romania 20% of 2,000 wells had nitrate levels above 23 mg/L as nitrate-N (Jedrychowski et al. 1997). Studies from other countries including China, Botswana, Turkey, Senegal, and Mexico report private well water levels that exceed the WHO guideline, in some instances at levels higher than 68 mg/L nitrate-N (World Health Organization 2004). While fertilizer is the main contributing factor in agricultural areas, nitrogen from human waste appears to be the most important source in urban areas lacking centralized water and sanitation systems. Although systematic information on nitrate levels in ground water in other parts of the world is more limited, empirical modeling approaches have indicated that users of shallow

wells in areas with high nitrogen inputs, well drained soils, and unconsolidated rocks are most at risk of consuming high-nitrate ground water (Nolan et al. 2002).

Methemoglobinemia

Ingested nitrate is reduced to nitrite which binds to hemoglobin to form methemoglobin (MetHb). Methemoglobinemia occurs when elevated levels of MetHb (exceeding about 10%) interfere with the oxygen-carrying capacity of the blood. Infants are particularly susceptible to developing methemoglobinemia for several reasons including their increased capacity to convert nitrate to nitrite and their lower levels of the enzyme cytochrome b5 reductase which converts MetHb back to hemoglobin. Methemoglobinemia in infants fed formula made with well water with high nitrate levels was first reported in 1945 by Comly (1945). The regulatory level for nitrate in drinking water supplies was determined after a survey of infant methemoglobinemia case reports in the United States indicated that no cases were observed at drinking water nitrate levels below 10 mg/L nitrate-N (Walton 1951). Since an estimated 22% of domestic wells in agricultural regions of the US exceed the nitrate MCL (U.S. Geological Survey, unpublished data), it is likely that significant numbers of infants are given water containing more than 10 mg/L nitrate-N. Nevertheless, few cases of methemoglobinemia have been reported since the MCL was promulgated.

The risk of methemoglobinemia among infants depends on many factors other than the ingestion of nitrate in drinking water. Some foods and medications contain high levels of nitrate (Sanchez-Echaniz et al. 2001). Enteric infections, potentially caused by fecal bacteria contamination in wells, may lead to the endogenous production of nitrite, as

evidenced by numerous published reports of infants with diarrhea and methemoglobinemia, but no apparent exposure to exogenous methemoglobin-forming agents (Charmandari et al. 2001; Hanukoglu and Danon 1996; Levine et al. 1998; Wennmalm et al. 1993). The consumption of antioxidants such as vitamin C appears to be a protective factor. Finally, polymorphisms in the activity of cytochrome b5 reductase may mediate the effect of ingested nitrate or endogenously produced nitrite (Gupta, et al., 1999).

Studies that have examined the relationship between nitrate levels in drinking water and MetHb levels in infants have produced mixed results (Environmental Protection Agency 1991). The few experimental studies are largely negative; however, most of these studies evaluated low levels of drinking water nitrate and included few infants. Co-factors such as diarrhea and respiratory diseases have been reported to increase MetHb levels (Shuval and Gruener 1972; Shearer et al.1972). An epidemiologic study in South Africa (Super et al. 1981) found an increase in MetHb levels in infants fed water with nitrate above 20 mg/L nitrate-N; however, clinical methemoglobinemia was rarely found. A protective effect of vitamin C intake on MetHb was noted (Super et al. 1981). More recently, a retrospective nested case-control study in Romania found an association between nitrate exposure from drinking water and clinical methemoglobinemia, however there was also some evidence of an association with diarrheal disease (Zeman et al. 2002). Gupta and colleagues (1999) found cytochrome b5 reductase activities to be higher among those consuming water with high nitrate levels, indicating a level of adaptation to the consumption of high nitrate waters.

Recently, the role of nitrate exposure alone in causing methemoglobinemia has been questioned (Avery 1999; Fewtrell 2004; Hanukoglu and Danon 1996). Clearly, there is a need to better understand the interaction of factors that lead to methemoglobinemia in order to assess the relative importance of each factor, and to identify the conditions under which exposure to nitrate in drinking water poses a risk of methemoglobinemia.

Nitrate intake and endogenous formation of N-nitroso compounds

Nitrate is a precursor in the formation of N-nitroso compounds (NOC), a class of compounds, which are genotoxic and most of which are animal carcinogens. In the human body, nitrate is a stable, inert compound that cannot be metabolized by human enzymes. However, the nitrate-reducing activity of commensal bacteria may convert nitrate into nitrite and other bioactive nitrogen compounds that affect physiological processes and human health. After ingestion, nitrate is readily absorbed from the upper gastrointestinal tract. Up to 25% is actively excreted in saliva, where about 20% is converted to nitrite by bacteria in the mouth (Spiegelhalter et al. 1976). This conversion can occur at other sites including the distal small intestine and the colon.

Under acidic conditions in the stomach, nitrite is protonated to nitrous acid (HNO_2), which in turn spontaneously yields dinitrogen trioxide (N_2O_3), nitric oxide (NO) and nitrogen dioxide (NO_2). NO is a bioactive compound known to play a role in vasodilatation and in defense against periodontal bacteria and other pathogens. N_2O_3, on the other hand, is a powerful nitrosating agent capable of donating NO^+ to secondary and tertiary amines to

form potentially carcinogenic N-nitrosamines (Leaf et al., 1989). Alternatively, HNO_2 can be protonated to H_2NO_2 which reacts with amides to form N-nitrosamides. At neutral pH, nitrite can be reduced by bacterial activity to form NO, which can react with molecular oxygen to form the nitrosating compounds N_2O_3 and N_2O_4. In addition to the acid-catalyzed and bacterial catalyzed formation of nitrosating agents, inducible nitric oxide synthase activity of inflammatory cells can also produce NO (Ohshima and Bartsch 1994). Together these three mechanisms of endogenous nitrosation are estimated to account for roughly 40-75% of the total human exposure to NOC (Tricker 1997). Other sources of human exposure include preformed NOC found in preserved meats and fish, beer, certain occupational exposures, and tobacco products (Tricker 1997).

Several studies support a direct relationship between nitrate intake and endogenous formation of NOC. High intake of drinking water nitrate (above the MCL) has been associated with an increased endogenous capacity to nitrosate proline (Moller et al. 1989; Mirvish et al. 1994). Also, populations with high rates of esophageal and gastric cancer were found to excrete high levels of N-nitrosoproline (Lu et al. 1986; Kamiyama et al. 1987). Nitrate intake at the acceptable daily intake level (3.67 mg/kg body weight, 0.84 mg/kg as nitrate-nitrogen) results in increased urinary excretion of NOC, particularly in combination with increased intake of dietary nitrosatable precursors (Vermeer et al. 1998). However, a Canadian population exposed to nitrate below the acceptable daily intake level, showed no relationship between nitrate levels in drinking water and urinary nitrosamines (Levallois et al. 2000).

Factors that modulate endogenous nitrosation

Although there appears to be a consistent association between intake of high drinking water nitrate and endogenous nitrosation capacity, intake of dietary nitrate is less likely to increase nitrosation due to the presence of nitrosation inhibitors in vegetables, the major contributors to dietary nitrate intake (Bartsch et al. 1988; National Academy of Sciences 1981). Dietary compounds that inhibit endogenous nitrosation, include vitamin C which has the capacity to reduce nitrous acid to NO, and alpha-tocopherol which can reduce nitrite to NO. Several epidemiological studies reported no association or inverse associations between dietary nitrate intake and human cancers (Ward et al. 1996; Boeing 1991; Forman 1987), which may be due to the antioxidants and nitrosation inhibitors in nitrate-containing foods (Bartsch et al. 1988). Inhibitory effects on nitrosation have also been described of betel nut extracts, ferulic and caffeic acid, garlic, coffee and green tea polyphenols (Stich et al. 1984). Also, non-dietary factors like the use of chlorhexidine containing mouthwashes can influence the endogenous nitrosating capacity, (van Maanen et al. 1998).

Apart from the level of nitrosating agents, the level of nitrosatable precursors in the diet, which come predominantly from meat and fish, is a crucial factor in endogenous nitrosation. Dietary intakes of red and processed meat are of particular importance in the formation of fecal NOC (Bingham et al.1996; 1999; 2002; Cross et al. 2003; Haorah et al. 2001). Higher consumption of red meat (600 versus 60 g/day) (but not white meat) resulted in a 3-fold increase in fecal NOC levels (Bingham et al. 1996). Colon cancer incidence is most consistently associated with consumption of red meat (beef, lamb and

pork), but not with poultry and fish (Bingham 1999). Dietary supplementation of a low red meat diet with either haem iron or inorganic iron demonstrated that particularly haem was capable of stimulating endogenous nitrosation (Cross et al. 2003), thereby providing a possible explanation for the differences in colon cancer risk between red and white meat consumption. Additionally, it may be proposed that this linkage may be stronger for processed than for fresh meat because of the higher NOC and NOC precursor levels in processed meat.

Endogenous nitrosation can also be stimulated by inflammatory and other medical conditions. For instance, patients with bilharzia have an increased bladder cancer risk that is associated with increased urinary levels of nitrite and volatile nitrosamines. These are most likely generated by the reaction of inflammation-derived NO with amines present in the urine (Tricker et al. 1989). Also inflammatory bowel disease has been related to both increased nitrosation and cancer risk (Lashner et al. 1988). During inflammatory bowel disease, increased inducible nitric oxide synthase activity can produce excess NO, which is oxidized to nitrogen oxides and nitrite, which in turn react with nitrosatable precursors in colonic contents to produce NOC. Indeed, ulcerative colitis patients showed increased levels of inducible nitric oxide synthase in the colonic mucosa (Kimura et al. 1998) and of NO and nitrite in the colonic lumen (Roediger et al. 1990, Lundberg et al. 1997). Increased levels of fecal NOC have been found in patients with inflammatory bowel disease and in mice with chemically induced colitis, (de Kok et al. 2005; Mirvish et al. 2003).

Health effects associated with drinking water nitrate

Cancer

NOC are potent animal carcinogens, inducing tumors at multiple organ sites including the esophagus, stomach, colon, bladder, lympatics, and hematopoietic system (Bogovski and Bogovski, 1981). NOC cause tumors in every animal species tested and it is unlikely that humans are unaffected (Lijinsky 1986). The number of well-designed epidemiologic studies with individual exposure data and information on nitrosation inhibitors and precursors are few for any single cancer site, limiting the ability to draw conclusions about cancer risk.

Most studies have been ecologic in design, linking incidence or mortality rates to drinking water nitrate levels at the town or county level. The early studies focused on stomach cancer mortality and most used drinking water nitrate measurements concurrent with the time period of cancer mortality. Results were mixed, with some studies showing positive associations, many showing no association and a few showing inverse associations (Cantor 1997). Recent ecologic studies of stomach cancer in Slovakia, Spain, and Hungary with historical measurements and exposure levels near or above the MCL have found positive correlations with stomach cancer incidence or mortality (Gulis et al. 2002; Sandor et al. 2001; Morales-Suarez-Varela et al. 1995). Two studies included other cancer sites. In Slovakia, incidence of NHL and colon cancer was significantly elevated among men and women exposed to public supply nitrate levels of 4.5-11.3 mg/L nitrate-N (Gulis et al. 2002); there was no association with bladder and kidney cancer incidence. In Spain there was a positive correlation between nitrate levels in public supplies and prostate cancer

mortality, but no relation with bladder and colon cancer (Morales-Suarez-Varela et al. 1995).

In the past decade, several case control and cohort studies have evaluated historical nitrate levels in public water supplies (largely below 10 mg/L nitrate-N) and risk of several cancers (Table 1). Some studies evaluated factors affecting nitrosation such as vitamin C intake. A cohort study of older women in Iowa (USA) (Weyer et al. 2001) found a 2.8-fold and 1.8-fold risk of bladder and ovarian cancers, respectively, associated with the highest quartile (>2.46 mg/L nitrate-N) of the long-term average nitrate levels at the current residence. They observed significant inverse associations for uterine and rectal cancer and no significant associations for NHL, leukemia, colon, rectum, pancreas, kidney, lung, and melanoma. Case-control studies of bladder (Ward et al. 2003), brain (Ward et al. 2004), colon and rectum (De Roos et al. 2003), and pancreas cancer (Coss et al. 2004) in Iowa found no association between cancer risk and average nitrate levels over almost 30 years. Each study evaluated the interaction between nitrosation inhibitors or NOC precursors and nitrate intake from drinking water. For colon cancer, there was a significant positive interaction between 10 or more years of exposure above 5 mg/L nitrate-N and both low vitamin C and high meat intake, factors which are likely to increase endogenous NOC formation (De Roos et al. 2003).

A case-control study of NHL in Nebraska (Ward et al. 1996) found a significant positive association between the average nitrate level in public water supplies over about 40 years and risk among men and women. In the highest quartile of nitrate (4.0 mg/L nitrate-N),

risk was elevated two-fold. However, a recent study of NHL in Iowa with similar exposure levels, found no association (Ward et al. 2004). A case-control study of NHL in Minnesota (Freedman et al. 2000) with lower levels of nitrate found an inverse association among those with the highest level (>1.5 mg/L nitrate-N). Case-control studies in Nebraska (Ward et al. 2004) and Germany (Steindorf et al. 1994) found no association with long-term average nitrate levels in public water supplies and adult brain cancer. The Nebraska study found no evidence of an interaction with vitamin C intake. A case-cohort analysis of stomach cancer, within a cohort study in the Netherlands (van Loon et al. 1998) found no association with quintiles of water nitrate intake determined from public supply levels. Specific NOC are transplacental neurocarcinogens in animal studies. A study of childhood brain cancer measured nitrate levels in water supplies using dipstick measurements, often many years after the pregnancy (Mueller et al. 2001). Measured levels of nitrate and nitrite were not associated with risk; however, women in western Washington State, one of the three study centers, who used private wells as their drinking water source during the pregnancy had a significantly increased risk of brain cancer in their offspring.

Adverse Reproductive Outcomes

In 1961, Schmitz described a possible relationship between high maternal methemoglobin levels and spontaneous abortion. Since then, at least 10 studies have examined the association between drinking water nitrate and adverse reproductive outcomes. Table 2 summarizes these studies by location, study design, determination of water nitrate, and key findings. Few studies have been published regarding water nitrate and the outcomes of

spontaneous abortions, stillbirths, premature birth, or intrauterine growth retardation. Results of these studies have been inconsistent possibly indicating that there is no true effect of water nitrate on reproductive outcomes at the levels evaluated in these studies. Alternatively, the inconsistencies may be due to the differing time periods over which exposure was assessed, differing levels of water nitrate across studies, or differences in exposure to other co-factors.

Results of studies evaluating drinking water nitrate and congenital malformations in offspring are also mixed (Table 2). Four studies (Arbuckle et al. 1988; Brender et al. 2004a, Brender et al. 2004b, Croen et al. 2001, Dorsch et al. 1984) found positive associations between drinking water nitrate and congenital malformations, particularly malformations of the central nervous system, and specifically neural tube defects. In each of these studies, water nitrate levels associated with increased risk of these defects were below the MCL, although the 95% confidence intervals for some of the risk estimates were consistent with unity and varied by the source of water (groundwater, mixed, or surface). Two of these studies (Croen et al. 2001; Brender et al. 2004a) also examined dietary intake of nitrates and nitrates and neural tube defects and found minimal or no effect on risk. In a study of nitrosatable drug exposure and risk of neural tube defects (Brender et al. 2004a), drinking water nitrates and dietary nitrites/total nitrites substantially modified the risk associated with this drug exposure during the periconceptional period; higher levels of nitrates in food or water significantly increased the risk of neural tube defects if women were exposed to such drugs.

Other health outcomes

Animal studies suggest that nitrate at high doses can competitively inhibit iodine uptake and induce hypertrophic changes in the thyroid (Bloomfield et al. 1960). In a human biomonitoring study in the Netherlands, consumption of water with nitrate levels at or above the MCL was associated with thyroid hypertrophy (van Maanen et al. 1994) and genotoxic effects (van Maanen et al. 1996). Animal studies provide evidence that NOC can damage the pancreatic beta-cells (Longnecker and Daniels 2001). Three epidemiologic studies (Kostraba et al. 1992; van Maanen et al. 2000; Parslow et al. 1997) that were ecologic in design found a positive correlation between drinking water nitrate levels below the MCL and the incidence of type I childhood diabetes, although the association observed by van Maanen was not statistically significant. Other studies have found associations between water nitrate exposure and increased blood pressure (Poomeranz et al. 2000) and acute respiratory tract infections in children (Gupta et al. 2000).

Recommendations for future research

Experimental/human biomonitoring studies

Endogenous nitrosation in humans has been demonstrated in relation to drinking water nitrate ingestion at levels above the MCL. However, further studies are needed to determine the extent of endogenous nitrosation at intermediate drinking water nitrate levels (5-10 mg/L as nitrate-N) and to clarify the role of nitrate from water as compared with food sources. Furthermore, the role of precursors and modulators of NOC formation

should be more fully investigated. These future studies should be conducted among healthy individuals as well as individuals with medical conditions that increase endogenous nitrosation.

In view of the complex kinetics of NOC formation and the organ specificity of several of these compounds (Hodgson et al. 1980; Suzuki et al. 1999), more studies are needed to evaluate the relationship between nitrate intake and formation, metabolism and excretion of NOC. Ideally, a physiologically based pharmacokinetic model should be developed as previously recommended (National Research Council 1995) in order to predict exposure to NOC from all sources of nitrate exposure (exogenous and endogenous), nitrite intake, the transformation of nitrate into nitrite, and anti-oxidant intake. However, this will require additional data on the formation of individual NOC as well as their respective toxicological characteristics. The results of these investigations will reveal the value of different markers of NOC exposure in future epidemiological studies. Future studies linking NOC exposure to early makers of effect or to the actual disease will clarify the role of endogenous nitrosation and NOC-exposure as etiologic factors.

Because many NOC require α-hydroxylation by CYP2E1 for bioactivation and to form DNA adducts, it is important to investigate the influence of polymorphisms in the gene encoding for this enzyme. One study found that specific variants in this gene are linked to increased rectum cancer risk, particularly in subjects with high intake of red and processed meat, who are exposed to increased levels of NOC (Le Marchand et al. 2002). Moreover, gene expression levels of human CYP2E1 were found to be related to cytotoxicity and

DNA damage by nitrosamines in pancreatic beta-cell lines suggesting that such gene environment interactions are also relevant in type I diabetes (Lees Murdock et al. 2004). These promising lines of research point to a possible interaction between drinking water nitrate exposure and gene expression of and/or genetic variation in CYP2E1 which may also influence the risk of several adverse health outcomes associated with nitrate exposure.

Epidemiologic studies

Methods need to be developed and validated to improve estimates of current and historical exposure to nitrate via foods and water, particularly for populations served by private wells which are less likely to be routinely monitored. Future epidemiologic studies should integrate i) exposure assessment for nitrate intake from drinking water, nitrate and nitrite intake from the diet, and amines and amides from dietary and drug sources, ii) endogenous exposure to NOC by analysis of relevant biological media (e.g. saliva, urine and/or feces) and iii) reliable health risk markers (e.g. biomarkers of genotoxicity) or diagnosis of actual disease.

Future studies should include populations with well-characterized long-term exposures including those who use private wells which can have higher nitrate levels than public supplies. With the increasing availability of public water supply monitoring data (many US states have almost 40 years of measurements) further detailed exposure assessment of populations using public supplies is also feasible. Drinking water contaminants that may co-occur with nitrate, such as agricultural pesticides, should also be evaluated. Geographic-based modeling efforts to predict the probability of high nitrate concentrations

in groundwater using information on nitrogen inputs from agricultural and urban sources (Nolan et al. 2002) is a promising approach for estimating drinking water nitrate exposure for the population using private wells.

Additional studies of drinking water nitrate and cancer are needed to follow up on the suggestive positive findings to date and to evaluate other cancer sites for which human biomonitoring studies and/or animal studies suggest endogenously formed NOC may play a role. Studies of reproductive outcomes should address the exposure period most relevant for the specific outcome of interest. Maternal residential mobility between conception and birth may lead to misclassification of exposure if the water source at birth is used in studies of spontaneous abortions and congenital malformations. Studies need to be of sufficient size to allow for examination of specific defects rather than groups of defects by system; "lumping" different defects together might mask associations. More research is needed on the relation between water nitrate and the reproductive outcomes of spontaneous abortion, fetal death, premature birth, and intrauterine growth retardation.

In the design and analysis stage, future epidemiological studies should take into account factors that modulate endogenous nitrosation, as discussed above, in order to be able to evaluate potential interactions of water nitrate intake with these factors, thus providing stronger evidence for or against an association. In particular, studies of susceptible populations may be fruitful and epidemiologic studies should be designed with sufficient power to evaluate risk among potentially susceptible subgroups. Such populations include patients with different forms of chronic inflammation (such as inflammatory bowel

disease), patients infected with nitrate reducing bacteria (such as in periodontal disease), those with a systematic low intake of vitamins and other known nitrosation inhibitors, or those with a history of high incidence of potentially NOC-related diseases. An example of a population that fits the latter two categories are the people of Linxian County in China, known for their persistently low intake of several micronutrients and high risk of esophageal cancer (Blot et al.1993). Apart from the fact that such studies will provide better understanding of the risks associated with nitrate in drinking water and other NOC precursors, these are also the populations that are likely to benefit the most from preventive measurements that can be taken based on the results of these investigations.

Conclusions

Adverse health effects from drinking water nitrates are most likely the result of a complex interaction of the amount of nitrate ingested, the concomitant ingestion of nitrosating co-factors and precursors, and medical conditions of the host which may increase nitrosation. Furthermore, these effects may be attenuated by inhibitors of endogenous nitrosation such as vitamin C and alpha-tocopherol. It is recommended that future studies take into account such complexities in understanding the relation between drinking water nitrates and cancer, adverse reproductive outcomes, and other health outcomes.

A number of authors (Avery 1999; L'hirondel and L'hirondel 2002) have questioned the importance of nitrate in drinking water as a risk factor for methemoglobinemia and have suggested that the current nitrate standard might be safely raised to 15-20 mg/L nitrate-N

with no increase in methemoglobinemia cases. A better understanding of the conditions under which nitrate in drinking water poses a risk of methemoglobinemia is clearly needed, particularly in light of recent cases of methemoglobinemia associated with well water levels between 20-30 mg/L nitrate-N (Knobeloch et al. 2000). Most importantly, the role of nitrate as a risk factor for cancer and adverse reproductive outcomes needs to be more thoroughly explored before changes to nitrate water quality standards are considered.

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Table 1. Analytic epidemiologic studies of drinking water nitrate and cancer.

First Author (year) Country	Study design (case-control, cohort) Regional description	Years of cancer ascertainment	Exposure description[1]	Cancer sites included	Summary of findings
Coss (2004) USA	Population-based case control Incidence Iowa	1986-89	Average nitrate level in public supplies 1960-87 (highest quartile >2.8 mg/L); Years of exposure ≥ 7.5 and 10 mg/L	Pancreas	No significant associations with quartiles of average nitrate or number of years ≥7.5 or 10 mg/L
DeRoos (2003) USA	Population-based case-control Incidence Iowa	1986-89	Average nitrate level in public supplies 1960-1987 categorized into 4 levels (lowest: ≤1.0; highest: >5mg/L); Years of exposure >5 and >10 mg/L	Colon Rectum	No association with average level, years >5 and 10 mg/L; Significantly elevated risk among subgroups with below median vitamin C intake or above median meat intake and 10 or more years >5 mg/L
Freedman (2000) USA	Population-based case-control Incidence Minnesota excluding 4 largest Cities	1980-82	Average nitrate level in public water supplies 1947-1980 (157 towns) categorized into 3 levels: ≤0.5, >0.5-≤1.5, >1.5 mg/L)	Non-Hodgkin's lymphoma	No increase risk with increasing exposure level. OR for >1.5 mg/L (3 cases, 4 controls) was 0.3 (95% CI 0.1-0.9).
Mueller (2001) USA	Population-based case-control 19 counties in San Francisco, California area and western Washington State	1984-1990	Water source (private well, public supply) during pregnancy; dipstick measurements of nitrate and nitrite for those still living at residence during pregnancy	Childhood brain	No overall association with water source. Well use in western Washington State increased risk (OR=2.6, 95% CI 1.3-5.2); well use in Los Angeles inversely associated with risk (OR=0.2, 0.1-0.8)
Steindorf (1994) Germany	Population-based case-control Incidence Rhein-Neckar-Odenwald area	1987-88	Nitrate levels in municipal supplies after 1970 (highest quartile: >5.7 mg/L)	Brain	No association with average nitrate level

Van Loon (1998) Netherlands	Prospective cohort Incidence	1986-92	Nitrate intake from public supplies in 1986 and intake of tap water (quintiles; mean level in highest quintile 3.7 mg/day)	Stomach	No association with quintiles of water nitrate intake (highest quintile: RR=0.88)
Ward (1996) USA	Population-based case-control Incidence 66 counties in eastern Nebraska	1983-86	Average nitrate level in public water supplies 1945-early 1980s categorized into quartiles (lowest: <1.6; highest: ≥4.0 mg/L); Ever exposure ≥ 10 mg/L	Non-Hodgkin's lymphoma	Significant positive trend with increasing quartiles: OR highest quartile=2.0 (95% CI 1.1-3.6)
Ward (2003) USA	Population-based case-control Incidence Iowa	1986-89	Average nitrate level in public water supplies 1960-1987 (highest quartile men: 3.1 mg/L; women: 2.4 mg/L); Years of exposure ≥10 mg/L	Bladder	Inverse association with quartiles of average level among men; no association among women. Similar results for years ≥10 mg/L
Ward (2004) USA	Population-based case-control Incidence 66 counties of eastern Nebraska	1988-93	Average nitrate level in public water supplies 1960-1986;	Brain (gliomas)	No association with quartiles of the average nitrate level
Weyer (2001) USA	Prospective cohort Incidence Iowa	1986-98	Average nitrate level (1955-1988) in public water supplies for residence at enrollment (highest quartile: >2.46 mg/L)	Non-Hodgkin's lymphoma, Leukemia, Colon, Rectum, Pancreas, Kidney, Bladder, Breast, Ovary, Uterine corpus, Lung and bronchus, Melanoma	Positive associations with average nitrate level for bladder (highest quartile OR=2.83) and ovary (OR=1.84) and inverse associations for uterus (highest quartile OR=0.55) and rectal cancer (OR=0.47).

[1] Nitrate levels presented in the original publications as mg/L nitrate were converted to mg/L nitrate-nitrogen

Table 2. Studies of the relation between drinking water nitrate[1] and reproductive outcomes

Reference, study population, study design	Measurement of water nitrate	Reproductive outcome	Reported findings
Aschengrau et al., 1989 Massachusetts (USA) residents Hospital case-control study	Matched maternal residence at pregnancy outcome to results of tap water samples	Spontaneous abortions through 27 weeks of gestation	OR of 0.5 for SB with exposure to water nitrate levels of 0.1-5.5 mg/L relative to non-detectable levels
Grant et al., 1996 Indiana (USA) Cluster investigation	Wells tested for nitrates after cluster reported	Spontaneous abortions	Water nitrate above USEPA MCL for women with SB
Aschengrau et al., 1993 Massachusetts (USA) residents Hospital case-control study	Matched maternal residence during pregnancy or outcome to results of tap water samples	Congenital anomalies stillbirths, neonatal deaths	Neither stillbirths or congenital anomalies associated with detectable levels of water nitrate (0.2-4.5 mg/L); small positive association between water nitrates and neonatal deaths.
Super et al., 1981 South West Africa Cross-sectional study	Water sample taken from well used at time of home visit	Spontaneous premature labor Size of infant at birth	No association between water from high nitrate regions and prematurity or size of infant
Bukowski et al., 2001 Prince Edward Island Canada Case-control study	Residential postal code at time of delivery linked to nitrate level exposure map	Intrauterine growth retardation Premature birth	Dose-response relation between nitrate level and ORs for IUGR and prematurity
Scragg et al., 1982 Dorsch et al., 1984 South Australia Case-control study	Address at delivery linked to sources of water and data on nitrates	Congenital malformations	Elevated OR for any congenital malformation (2.8); malformations of the CNS (3.5); musculoskeletal system (2.9) if primarily drank groundwater. Elevated ORs for congenital malformations associated with nitrate levels ≥ 5 mg/L, relative to nitrate levels < 5 mg/L.

Reference, study population, study design	Measurement of water nitrate	Reproductive outcome	Reported findings
Arbuckle et al., 1988 New Brunswick, Canada Case-control study	Collected and analyzed a water sample at maternal residence at time of index birth	Congenital malformations of the central nervous system	OR of 2.3 for CNS malformations with exposure to nitrate 26 mg/L relative to baseline of 0.1 mg/L
Ericson et al., 1988 All deliveries in Sweden Case-control study	Earliest known maternal address linked to water nitrate results	Neural tube defects	Average water nitrate similar between cases and controls
Croen et al., 2001 California (USA) Case-control study	Linked peri-conceptional addresses to water companies and databases	Neural tube defects	Exposure to water nitrates > 45 mg/L associated with anencephaly (OR 4.0) but not with spina bifida; increased risks for anencephaly at water nitrate levels below USEPA MCL among groundwater drinkers only; dietary nitrate and nitrite not associated with NTDs
Cedergren et al., 2002 Ostergotland County, Sweden Retrospective cohort study	Linked peri-conceptional or early pregnancy address to water supplies using a geographic information system	Any congenital cardiac defect	Weak association (OR 1.2) between water nitrate ≥ 2 mg/L and cardiac malformations
Brender et al., 2004a Brender et al., 2004b Texas (USA) Counties along Texas-Mexico border Case-control study	Usual peri-conceptional drinking water source tested for nitrates	Neural tube defects	OR of 1.9 if water nitrates ≥ 3.52 mg/L; increased water nitrate associated with spina bifida (OR 7.8) but not with anencephaly (OR 1.0); slightly inverse relation between dietary nitrite, total nitrite intake and NTDs

[1] Nitrate units are mg/L as nitrate

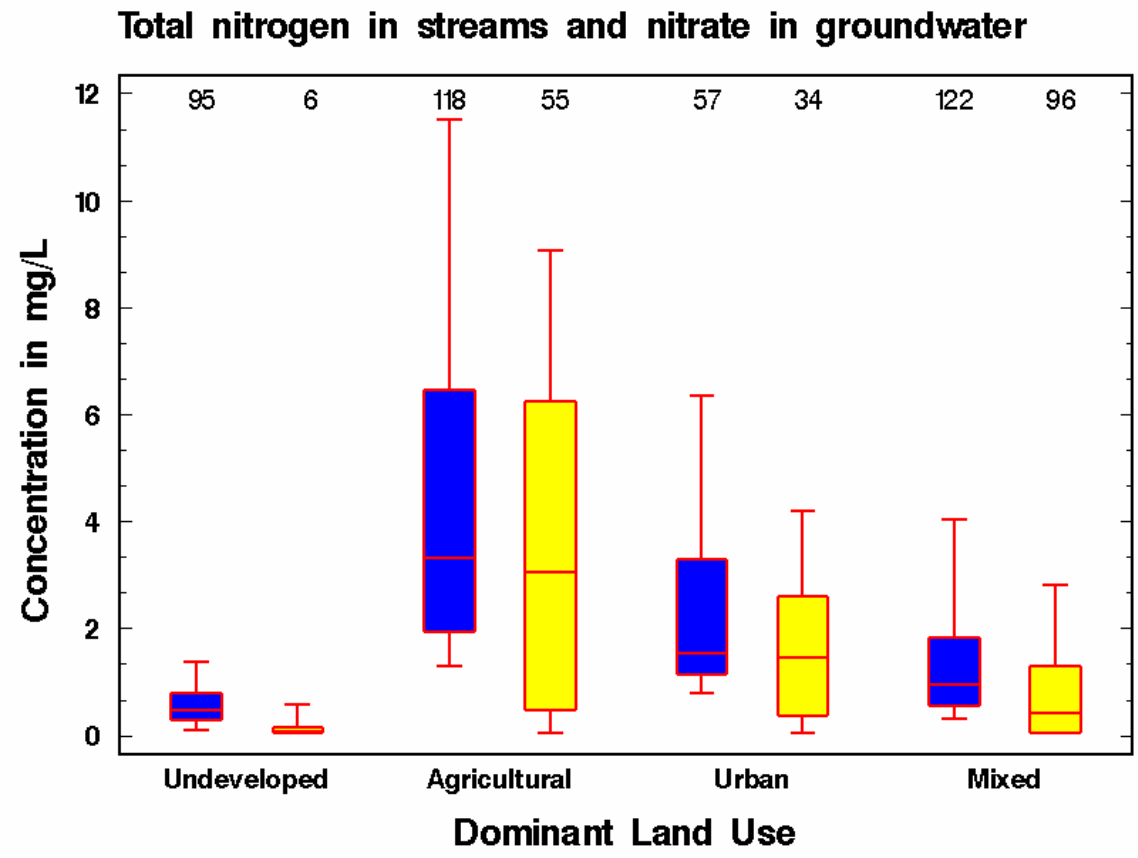

Figure 1. Total nitrogen in streams (blue bars) and nitrate-N in ground water (yellow bars) in agricultural, urban, mixed land use, and undeveloped areas of the United States.